PATENT COOPERATION TREATY

Rec'd PCT/PTO 19 JAN 2005

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| LBP1000PC00 | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/EP 03/07946 | 21.07.2003 | 19.07.2002 |
| International Patent Classification (IPC) or both national classification and IPC | | |
| C12N15/85 | | |
| Applicant | | |
| LONZA BIOLOGICS PLC. et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 17.12.2003 | 03.08.2004 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized Officer<br>Trommsdorff, M<br>Telephone No. +49 89 2399-7361 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/07946

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

1-20 as originally filed

**Sequence listings part of the description, Pages**

1-21 as originally filed

**Claims, Numbers**

1-8 received on 06.05.2004 with letter of 03.05.2004

**Drawings, Sheets**

1-3 as originally filed

**Sequence listing part of the description, pages:**

1-21, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-8 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-8 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1-8 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

## 1. Cited documents

D1: COCKETT M I ET AL: 'HIGH LEVEL EXPRESSION OF TISSUE INHIBITOR OF METALLOPROTEINASES IN CHINESE HAMSTER OVARY CELLS USING GLUTAMINE SYNTHETASE GENE AMPLIFICATION' BIO/TECHNOLOGY, NATURE PUBLISHING CO. NEW YORK, US, vol. 8, no. 7, July 1990 (1990-07), pages 662-667, ISSN: 0733-222X cited in the application

D2: PU H ET AL: 'RAPID ESTABLISHMENT OF HIGH-PRODUCING CELL LINES USING DICISTRONIC VECTORS WITH GLUTAMINE SYNTHETASE AS THE SELECTION MARKER' MOLECULAR BIOTECHNOLOGY, TOTOWA, NJ, US, vol. 10, 1998, pages 17-25,ISSN: 1073-6085

D3: BEBBINGTON C R ET AL: 'HIGH-LEVEL EXPRESSION OF A RECOMBINANT ANTIBODY FROM MYELOMA CELLS USING A GLUTAMINE SYNTHETASE GENE AS AN AMPLIFIABLE SELECTABLE MARKER' BIO/TECHNOLOGY, NATURE PUBLISHING CO. NEW YORK, US, vol. 10, no. 2, 1992, pages 169-175, ISSN: 0733-222X cited in the application

D4: WO 95 17516 A (HOLLIS GREGORY FRANKLIN ;MARK GEORGE E (US); MERCK & CO INC (US)) 29 June 1995 (1995-06-29) cited in the application

D5: US-A-5 891 693 (BEBBINGTON CHRISTOPHER ROBERT ET AL) 6 April 1999 (1999-04-06)

## 2. Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

2.1. The claims are directed to CHO cells transfected with different vector systems which allow enhanced expression of a heterologous gene of interest.
D4 describes a vector system containing a portion of the IgG2A locus and its use for homologous recombination in murine cells (examples 3-9). There is however no suggestion to use such a vector for higher expression levels in CHO cells.
Thus, claims 1-7 are novel over D4 (Art. 33(2) PCT).

2.2. Several prior art documents disclose the use of a glutamine synthetase (GS) gene as an amplifiable marker gene in CHO cells.
D1 discloses a vector system containing the glutamine synthetase gene and a gene of interest (the gene encoding the tissue inhibitor of metalloproteinases,

abbreviated TIMP) under the control of the human CMV promoter (p.666, material and methods) and shows that this system allows high level gene expression in CHO cells (Fig.2).
D2 discloses a dicistronic GS vector containing an IRES and shows that this vector system achieves higher expression levels than conventional vectors (Fig.3).
D3 teaches the use of GS as a selectable marker for high expression levels of an antibody driven by a human CMV-MIE promoter in myeloma cells.

Even though the glutamine synthetase gene was widely used for gene amplification, none of the available prior art documents discloses a GS based vector system containing the murine CMV promoter.
Since none of the specifically claimed CHO cells was disclosed in the prior art, the subject-matter of claims 1-8 is novel (Art. 33(2) PCT).

2.3. The claims are directed to CHO cells transfected with two different vector systems:
-on one hand a vector containing a portion from the murine IgG2A gene locus which is shown to enhance the human CMV promoter.
-on the other hand a vector containing two transcription units comprising a unit with a GS gene and a unit driven by the murine CMV promoter.
Since none of the prior art documents or combination of prior art documents suggests the transfection of such vectors into CHO cells and since the applicants show that both vector systems yield higher protein expression levels in CHO cells compared to standard vectors, claims 1-8 are also inventive (Art. 33(3) PCT).

Since the enhancing effect of the IgG2A locus has only been shown for the human CMV promoter and cannot be expected for all promoters, the claims should reflect the invention accordingly.

2.4. Note that the only common concept linking claims 1 and 8 is the fact that in both cases the vector systems used are engineered to enhance the expression of a heterologous gene of interest. Since such vectors are already known in the art and described in several of the above cited documents and since there is no other technical feature linking said claims, a lack of unity arises between claims 1-7 and claim 8 (Rule 13.1 PCT). The applicant should thus bear in mind that an objection for lack of unity of the application could be raised at a later time point, e.g. when

the application enters the regional phase.

2.5. The subject-matter of claims 1-8 is industrially applicable in the field of pharmaceutical industry (Art. 33(4) PCT).

## Claims

1. CHO cell transfected with an expression vector comprising a promoter that is active in CHO cells and that is driving expression of a recombinant product protein and further comprising a portion from the murine IgG 2 A gene locus DNA which portion is enhancing activity of said promoter.

2. CHO cell according to claim 1, characterized in that the vector further comprises a transcription unit encoding a selectable marker, preferably a glutamin synthetase (GS) marker.

3. CHO cell according to claim 1 or 2, characterized in the CHO cell is stably transfected.

4. Method of expressing a recombinant protein, comprising the steps of

   c. culturing a CHO cell transfected with an expression vector comprising a promoter active in CHO cells driving expression of a recombinant product protein and further comprising the murine IgG 2 A gene locus DNA or a DNA sequence variant or DNA fragment thereof which is enhancing activity of said promoter, and
   d. harvesting the product protein

5. Method according to claim 4, characterised in that the promoter is a strong viral promoter, preferably the hCMV promoter.

6. Method according to one of claims 4 or 5, characterised in that the IgG 2A gene locus portion does lack the natural immunoglobulin promoter.

7. Mammalian expression vector comprising at least a first transcription unit for a product gene which transcription unit is under the control of the mCMV promoter, and further comprising a second transcription unit comprising a glutamine synthetase (GS) marker gene.
8. CHO cell transfected with the vector of claim 7.